be explored. Darwin claims to have agonized over the evolution of the eye and much is made of the actual ease of its evolution in terms of dioptrics. Less often is it remembered that the hallmarks of any eye, its transparency and transduction mechanisms, depend on proteins (respectively crystallins and opsins) that evolved long before there were any eyes. And this molecular inherency underpins all biological complexity, even brains. Given these molecules, eyes (and nervous systems) are an inevitability.

Darwin's insights began with the behemoths of South America and the finches of Galapagos. Walcott in contrast was no biologist, but he knew at once that the Burgess Shale was wholly remarkable. For him the pressing urgency of description, not to mention his innumerable other commitments, never would allow him to reflect on what deeper implications this fauna might provide. But he lit the fuse, and just as the Darwinian formulation irrevocably destroyed any sort of Paleyean creationism, so I suggest Walcott will be seen as the one who one hundred years ago placed the first charges against the monolith. Darwin was right, but so too was Newton — in his way. Now we have a thrilling prospect of investigating fundamental principles that underpin the Darwinian story.

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Primer

Toxins and venoms

Edmund D. Brodie III

The macabre human fascination with natural toxins is age-old, but practical. From the eyes of newt and toads tossed in the cauldron of the witches of Macbeth, to the 'swamp adder' that serves as a near-perfect murder weapon in Doyle's *The Speckled Band*, poisonous creatures captivate people's imaginations precisely because they are so dangerous. Nonetheless, some of the most dramatic mysteries regarding natural poisons concern the evolutionary forces and processes that are responsible for the staggering diversity of compounds, delivery systems and organisms by which toxins and venoms take the stage.

Natural toxins can be found in virtually every major group of organisms, from fungi to mammals, from bacteria to birds. The actions of these poisons range from disrupting digestive processes to binding and blocking a single voltage-gated ion channel in a specific tissue. Some organisms seem to possess only a single toxic compound whereas others produce a whole cocktail of drugs with varying targets and effects. Some compounds are found in identical form in as many as five different phyla. A major challenge to understanding the biology of toxins is recognizing that many phenomena are artificially pooled under a single term.

The human vantage

Misleadingly, natural toxins and venoms are typically identified and categorized by their impact on humans. But this perspective has little to do with the ecological or evolutionary context of toxins. In most cases, the effect of a toxin on humans is an accidental byproduct of its primary function. Pit vipers, for instance, evolved in Southeast Asia and the New World, largely without interaction with large primates. Nonetheless, venoms produced by pit vipers have some of the most horrific effects known on human tissue and include compounds that are essentially digestive enzymes, usually referred to as hematoxins. Their immediate effect on humans, in addition to extreme pain, includes disruption of blood coagulation and the rapid digestion of soft tissues. The diversity and physiological effects of viper venoms cannot be understood from a human epidemiological perspective, because their effects on humans are secondary and accidental, and not the functions which these toxins were selected for.

The anthropocentric view of toxicity furthers obscures the biology of toxins and venoms because effects of a compound are context- or taxon-specific. Epidemiological work focuses on toxicity to mammals, using a mouse unit (MU) or LD50 as the metric by which to quantify the effect of a toxin. A MU is the quantity of a compound it takes to kill a 20 g mouse in a given time, whereas an LD50 is the dose that kills 50% of subjects in a given time. Such measures are problematic because they often return different values depending on the sex or strain of mouse used in the bioassay. Moreover, defining toxicity using a single species ignores that different species respond differently to the same compound. Botulinum toxin produced by the bacterium *Clostridium botulinum* is often cited as the most deadly natural poison known, with a human lethal dose of c. 0.7 µg, yet vertebrate carrion feeders, such as vultures, are resistant to the poison. Thus, toxicity is best defined operationally with respect to the specific taxa that the compounds have evolved in response to.

Why produce poisons?

Toxins and venoms serve a variety of functions. The three most common uses are predation or resource acquisition, defense and reduction of competition. The specifics of these functions and targets determine the shape of selection that modifies the compounds, and in turn the details of their consequences and severity. This is not to say that all toxic compounds found in nature bear their effect as a result of adaptive modification. In fact, many of the most extreme poisons may have accidental effects, or function as exaptations that arose for some other purpose or target and incidentally act as toxins in some ecological contexts.

The terms 'toxin', 'venom' and 'poison' are often loosely applied, contributing to confusion over function (Box 1). The distinction between toxin and venom is important because the natural selection pressures that drive

Box 1. Venom or toxin?

The labels 'venom' and 'toxin' refer to different modes of delivery of a poisonous compound, rather than the chemistry of the compound itself. This difference in delivery inexorably leads to disparate evolutionary paths and patterns.

Toxins (and poisons) are typically ingested or passively encountered. They may be collected in specialized structures but do not have any special mechanism of delivery into the body of another organism. For a toxin to enter the body of the recipient, it must be taken in by the recipient, either through ingestion or contact with a membrane. 'Toxin' normally refers to a singular molecular entity or compound with a specific action or binding site.

Venoms are housed and produced in specialized structures that are associated with a delivery device. These devices represent an amazing array of morphological convergence, from the hypodermic needle of a viper's fang to the lance of a wasp's sting, but all are capable of introducing the compound directly into the body of a recipient without the recipient's active participation. Venoms refer to a multipart blend of compounds that may have complex effects and target multiple tissues.

their evolution stem directly from their distinct functions. Toxins, generally, are not effective as mechanisms for subduing prey because they have to be taken up by the victim rather than delivered through an attack. The selection pressures on toxins are, therefore, less likely to directly drive immobilization or digestive action than those on venoms.

Foraging

Most venoms seem to have evolved to serve as foraging adaptations, with specific functions that influence the particulars of the compounds and their delivery. Immobilization of prey can be critical to reduce the risk of injury to the predator and speed the subjugation of prey. This is especially important for species foraging on large prey, or prey with significant defenses. Selection for immobilization favors venoms that are fast acting and directly influence mobility and coordination. For this reason, many venoms include a neurotoxic component that disrupts information transfer in nerves or muscle. In a striking example of functional convergence, both snakes and cone snails have evolved neurotoxic components, known as alpha-neurotoxins, that excite nicotinic acetycholine receptors in skeletal muscle. This class of toxins includes many different compounds with different chemical binding sites, but the general effect is the same — postsynaptic information transfer is blocked resulting in rapid paralysis. Other predators achieve much the same effect by blocking action potentials in nerves, usually by targeting voltage-gated ion channels. Mamba snakes immobilize prey with potassium channel blockers named 'dendrotoxins'. Scorpions, spiders, sea anemones, hymenopterans, and cone snails all produce different compounds that have the same potassium channel blocking pharmacological effect. Similar convergence (Figure 1) on target sites throughout the nervous system is found for other ion channels and receptors.

Other organisms require longer-term paralysis of their prey. Many solitary wasps provision their larvae with paralyzed but live prey that serve as the sole resource during development. Mud daubers, tarantula-hawks, cicada killers and others all attack and paralyze prey, lay an egg on the body, and seal the prey into a cell. This process typically takes many hours, during which the prey item is fully immobilized. In most cases, paralysis abates after a few days, but at this point the prey is enclosed in a cell with wasp larvae beginning to feed upon it. Venom components of these parasitoids typically include glutamate receptor blockers as well as ion-channel blockers to produce both quick acting and long-term paralysis. Behavioral components of envenomation are also critical to produce the necessary effects. One species of parasitoid wasp that hunts cockroaches uses three stings. The first sting, directed to the prothoracic ganglion, produces short-term paralysis, preventing the cockroach from defending itself. Subsequent stings target the subesophogeal ganglion and the brain and produce 20–30 min of grooming, followed by prolonged (2–3 weeks) hypokinesia. This integrated suite of behavioral and venom traits enables the wasp to lead the cockroach to a brood chamber and lay an egg upon it, after which the larvae feeds on the living prey until pupation.

Defense

Defensive toxins span a range of chemical categories and physiological activities, including channel-blocking neurotoxins, alkaloids that disrupt neuronal signaling, membrane-irritating terpenes and quinones, protease inhibitors that prevent digestion, and a variety of compounds that cause greater or lesser organismal insult. Some compounds elicit specific behaviors in predators, like the peptides in the skin mucus of *Xenopus* frogs that stimulate uncontrollable yawning and gaping that allow the frogs to crawl out of the mouth of snakes. The diversity of compounds and effects results from the rather simple selective scenario that drives their evolution — any compound that deters, delays or repels attack is favored. The specific toxins found in any particular lineage have more to do with the evolutionary history of a given group, or environmental sources of toxins, than with the specific effects they produce. In general, evolutionary convergence is less common among defensive compounds than among venoms.

One of the most puzzling paradoxes in the evolution of toxins is why organisms evolve to be deadly — contrary to venoms, for which deadly effects have a clear benefit. Extreme toxicity occurs repeatedly, from saturniid caterpillars to dart poison frogs. Selection favors the most-fit individuals, and those should be the ones that avoid predation. Killing an individual predator does not give an advantage over simply deterring one, especially if the prey has to be handled or eaten by a predator to deliver the poison. How, then, can we explain the evolution of deadly toxicity? In some sense, deadly toxins are accidental — small doses of death cap mushrooms (*Amanita phalloides*) are lethal to humans, but amatoxins certainly did not evolve to deter humans. The

answer may lie in counter-escalating arms races between predators and prey that drive the exaggerated evolution of toxicity in general, without resulting in deadly consequences to the primary selective agent. For example, some garter snakes of the genus *Thamnophis* are resistant to the sodium channel blocker tetrodotoxin found in the skin of rough-skinned newts (*Taricha granulosa*) (Figure 2). Coevolution with these resistant predators has driven the quantity of tetrodotoxin in newts in some places to a level sufficient to kill 10–20 humans or thousands of mice. At the same time, this level of toxicity barely impairs most garter snakes.

Venoms sometimes serve a defensive role, but this function is thought to be secondary. Despite the vernacular wisdom to the contrary, venomous snakes often bite defensively without injecting venom, the strike or bite alone being deterrent enough. Many venoms that have evolved as prey immobilizers also cause intense and immediate pain because of their effect on neuronal communication. Such compounds, common in hymenopterans, scorpions and jellyfish, have an obvious advantage as antipredator mechanisms. Selection to improve defensive function may have modified some components of venom blends to induce pain. Such a process might explain why some peptides produced by buthid scorpions selectively excite sodium channels in peripheral nerves, causing intense pain but not immobility. Some organisms may have secondarily evolved the ability to deliver toxins more actively (thus better fitting the definition of venom), as with the stinging hairs of *Lonomia* caterpillars that deliver hemorrhagic venoms that sometimes result in renal failure and death.

Competition

The ecological advantage of many compounds produced by bacteria, fungi, and plants involves competitive exclusion or manipulation. Allelopathic chemicals of plants are a well-known example of competition-reducing toxins. Juglone produced by black walnut inhibits plant respiration in many taxa, leaving the area around walnut trees relatively free of other potential competitors. Most of the toxins produced by bacteria that are dangerous to humans, including botulinum toxin, are thought to function as inhibitors of other bacteria that grow in diverse communities.

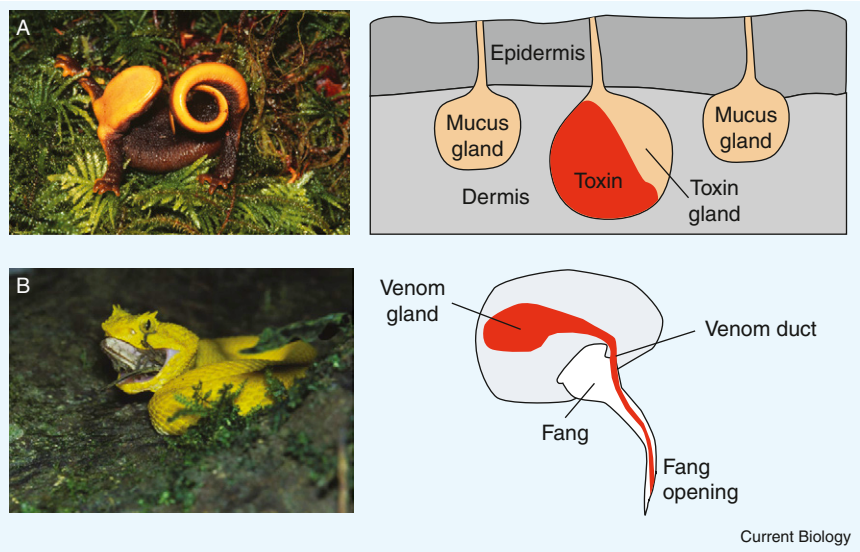

Figure 1. Delivery systems of toxins versus venoms.

(A) Toxins are typically stored in glands in the skin and delivered upon contact. The rough-skinned newt (*Taricha granulosa*) secretes tetrodotoxin when harassed and advertises its toxicity with both bright coloration and a defensive posture. (B) Venoms are usually produced in a gland connected to a specialized delivery apparatus, such as a fang or stinger, and are introduced directly into the body of another organism. Like all pit vipers, the eyelash viper (*Bothriechis schlegelli*) has extendable fangs that inject hematoxic compounds into prey to immobilize and speed digestion of their quarry (photos: E.D. Brodie III).

Sources of toxins and venoms

Though exceptions abound, venoms are most commonly produced by the organisms that possess them, while toxins are often sequestered from an outside source or modified from external building blocks; plants, fungi, and bacteria are all common producers of poisonous chemicals, whereas insects and vertebrates usually rely on others to do the work.

Venoms are often produced in specialized glands or other structures that are anatomically connected to delivery machinery (fangs, stingers or harpoons). For many components of venom mixtures in snakes, mollusks, hymenopterans and arachnids, researchers have been successful inlinking specific genes to the production of peptides and other compounds. Phylogenetic comparisons of toxin genes in reptiles, arthropods, and cephalopods, have each demonstrated ancient origins of a venomous ancestor as well as considerable convergence in the classes and structure of venom components. One interpretation of these findings is that ecologically important venom evolved early in the radiation of lizards and persists in many more lineages of reptiles than previously appreciated. Similarly, octopus, squid and other cephalods share similar biochemical compositions of their venom blends, suggesting both a venomous ancestor of the group and biased evolution of the specific components of venom.

Some groups seem unable or at least less likely to produce their own toxins. Insects, such as monarch and swallowtail butterflies, sequester toxins from host plants they fed on as larvae. Compounds such as the cardenolides of milkweeds or aristolochic acid of pipevines vary quantitatively among plants, and this variation generates differences in toxicity of the individual insects that feed on them. Sequestered toxins can become the linchpin for evolutionary trajectories of whole communities of insects. In eastern North America, as many as eight species of sympatric butterfly have evolved to resemble pipevine swallowtails because of their toxicity. In other species, exogenous toxins are even used as nuptial gifts — male *Utetheisa ornatrix* moths deliver a dose of sequestered pyrrolizidine alkaloids with their sperm that almost instantly renders females distasteful.

Plants are not the only source of sequesterable toxins. The poisons concentrated in the skin glands of

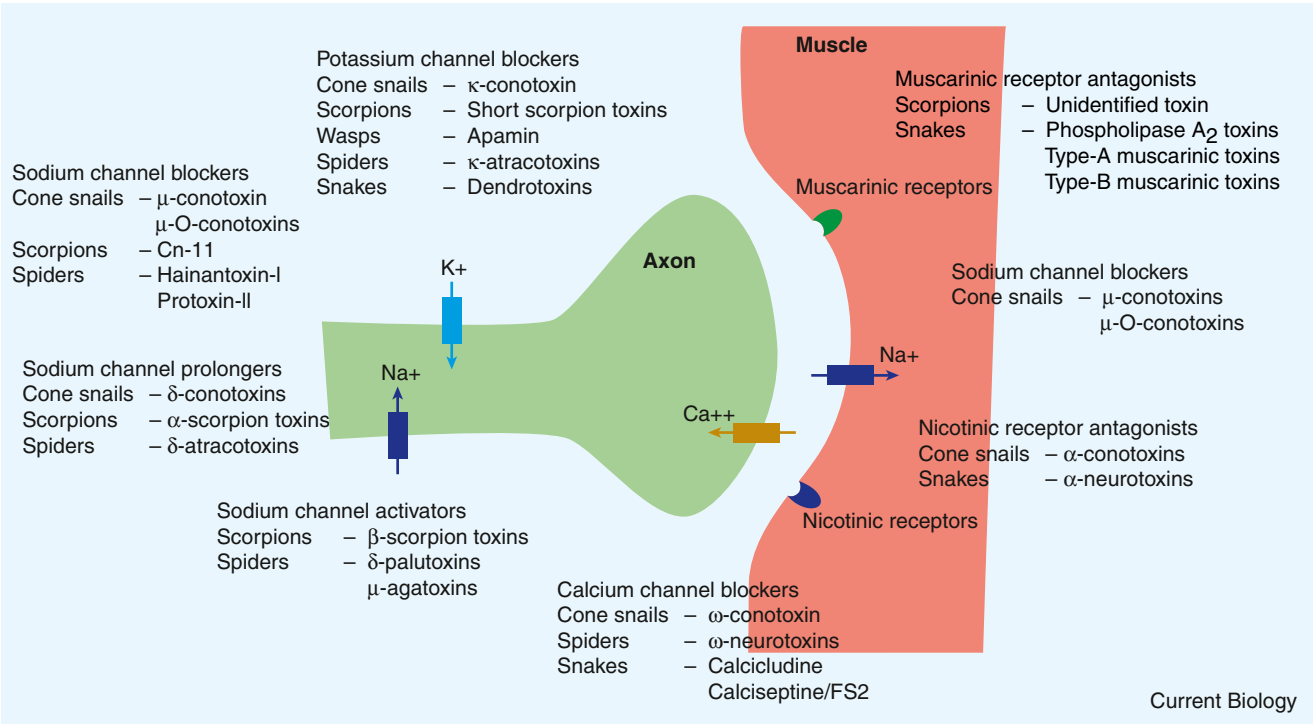

Figure 2. Functional convergence of neurotoxic venom components.

Many compounds used as venoms by different taxa disrupt pre- or postsynaptic communication in nerves and muscles. Although the compounds themselves vary greatly, they bind to the same target sites and result in common pharmacological effects. Modified after Fry *et al.* (2009).

dendrobatid frogs represent a broad range of neurotoxins. The specific compounds and their mixtures vary among species, and even within populations. Recent work correlated toxin profiles with the proportions of various millipedes and ants that produce the alkaloids found in frogs — explaining why these frogs lose their toxicity in captivity. In an astonishing case of evolutionary convergence, Madagascan frogs of the genus *Mantella*, which share ecology, color pattern and toxicity with the unrelated dendrobatids, also sequester toxins from arthropod prey, including at least nine identified alkaloids that are also known from dendrobatids. The arthropod sources of these toxins in the New and Old Worlds are essentially unrelated, indicating convergence of both toxin production and sequestration.

Other organisms may acquire toxicity through symbionts. The grass fescue is commonly infected with the endophytic fungus *Acremonimum coenophialum*, which produces an indole alkaloid with vasoconstrictive properties. Infection with the fungus reduces herbivory and may directly enhance growth. It is unclear whether this symbiosis arose through selection for toxicity in fescue, or whether the fungus began as a parasite that produced toxin for self-defense and inadvertently protected its host.

Symbiotic sources may be the most likely explanation for the wide distribution of other compounds in many different taxa. Tetrodotoxin (TTX) is known from no fewer than five phyla and some 20–30 species. It is almost unimaginable that so many taxa could have independently evolved an identical toxic compound for which no biosynthetic pathway is known. This led some to suspect a bacterial origin, and indeed TTX-producing bacteria have been cultured from some of the marine animals that are tetrodotoxic, but never from the terrestrial taxa. Nevertheless, each of the tetrodotoxic animal species must have evolved resistance to its channel-blocking effects, so it is clear that evolutionary convergence happens at some level.

Evolutionary patterns

There are two major, seemingly contradictory, themes in the evolution of toxins and venoms: on the one hand, evolutionary convergence of both toxins and venoms is widespread. On the other hand, venoms can exhibit some of the most rapid evolutionary divergence and variability of any category of proteins.

Convergence is the quintessential example of evolution finding similar solutions to selective challenges. This theme plays out in all aspects of venom and toxin biology, from acquisition and production, to chemical structure and pharmacological action. However, observing the same compound in diverse lineages does not always imply evolutionary convergence.

Venoms, more so than toxins, exhibit a degree of structural convergence across deep phylogenetic splits that suggests they evolve under a degree of functional or productive constraint (Figure 1). Many of the protein classes common in reptile venoms also have been recruited in the diverse cephalopod venoms. These proteins include a wide range of structures and actions, but some families of proteins are noticeably absent from venoms, including globular enzymes, transmembrane and intracellular proteins. Whether this convergence in chemical structure arises because of selective constraints in the pharmacological activity of venom, or

Figure 3. A cone snail.

Conotoxins of cone snails (including *Conus miliaris,* shown here) show evidence of exceptional evolutionary diversification in areas without competitors, suggesting ecological release in venom evolution (Photo: Thomas Duda).

through genetic constraints that limit the diversity of classes produced or recruited through evolution is not yet clear.

At the same time, rapid evolutionary diversification and variability is the hallmark of venom evolution. Marine cone snails (*Conus*) are predatory mollusks that fire a venomous harpoon that almost instantly immobilizes target prey (Figure 3). These darts carry an astonishingly diverse blend of neurotoxins that block ion channels or disrupt neuronal receptors. Molecular genetic analysis demonstrates that these compounds are under strong diversifying selection. The rate of protein evolution in conotoxin genes is three to five times higher than the highest rates observed for other proteins. There is evidence for gene duplication and diversification leading to the radiation of toxin forms. Similar rapid diversification is also observed for snake venom proteins. In both cases, it is assumed that strong selection to subdue prey is driving such fast evolution. Cone snails tend to feed on a relatively limited range of prey within populations, but the group as a whole feeds on many groups of invertebrate and vertebrate prey. Snails that feed on different prey types diverge in venom blends, but the biochemistry of conotoxins is much more diverse than snail diets (an individual snail may have 50–200 distinct toxic components). Geographic variation in venom composition also correlates with diet diversity in some vipers. Among species of elapids (cobras and their relatives), the three-fingered neurotoxins diversify rapidly as do diets of the venom producers. These examples all suggest that an arms race matching toxicity and resistance between predator and prey drives diversification of venoms. However, functional analyses that would more solidly confirm the existence of this process have yet to be conducted.

Conclusions

The compounds that we recognize as toxins and venoms span an enormous diversity of pharmacological and ecological functions. The selection pressures driving the modification of venoms and toxins are fundamentally different from those of other proteins, and result in somewhat different evolutionary dynamics. Toxins often appear convergent because externally available compounds are used for defense, whereas venoms seem to evolve toward a rather constrained set of target functions and classes of compounds. At the same time, venoms diversify within lineages at least as fast as any known group of proteins. With more experimental tools for more diverse groups of organisms, we will begin to discover what general evolutionary pressures and constraints shape the landscape of natural poisons.

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Ants use the panoramic skyline as a visual cue during navigation

Paul Graham[1,*] and Ken Cheng[2]

Foragers of many ant species learn long, visually guided routes between their nest and profitable feeding grounds [1–3]. The sensorimotor mechanisms underpinning the use of visual landmarks are much studied [3], but much less is known about how ants extract reliable visual landmark information from a complex visual scene. For navigation, useful visual information should be reliably identifiable across multiple journeys in differing lighting conditions, and one such robust source of information is provided by the skyline profile generated where terrestrial objects contrast against the sky. Experiments with ants and bees [4–6] suggest that insects might use directional information derived from the skyline, and in the work reported here, we explicitly tested this hypothesis. Ants were trained to shuttle between their nest and a feeder. We then recreated the skyline profile as seen from the feeder using an artificial arena with variable-height walls. Ants returning from the feeder were captured near their nest entrance and released in the arena. Ants followed the direction given by the artificial skyline when it was aligned with their habitual homeward compass direction or rotated by 150°. This result indicates that a crude facsimilie of a skyline can functionally mimic the natural panoramic scene.

We established an open access feeder 5 m from a *Melophorus bagoti* nest (Figure 1A) and the retinal elevation of the panoramic skyline (Figure 1B) from the feeder location was measured at 15° azimuthal intervals. At a distant test field an arena, 1 m in radius, was created using black plastic sheeting (Figure 1C). The height of the black plastic wall was varied so that from the centre of the arena the retinal elevation of the artificial